

## Alternative treatments in wound healing in animals

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### **Abstract**

*Treating wounds on a patient is a daily work for veterinarians in small animals practice. Wounds are not so simple to treat as we might think. It is important to assess the correct wound category in order to apply the right treatment. Antibiotics became challenging due to the worldwide problem of antibiotic resistance and so, alternative treatments started to emerge. In human medicine, people want to receive treatments which are the most natural as possible. Nowadays, Phytotherapy became more popular the last few years, even in veterinary medicine. Sea buckthorn is an ancient plant, very well-known remedy, since the dawn of time for its beneficial effects on the organism. In this study, we evaluated the effect of sea buckthorn oil on wound healing by second intention on rabbits, in comparison with Honeyderm, a product that is already on the market for veterinary medicine. The two products were applied on the same type of wounds but in different quantity per day and in comparison with a control one using saline solution. Histological examination highlighted better results in wounds treated with sea buckthorn oil. The cutaneous defects were filled with an abundant amount of immature fibrous tissue composed of large number of fibroblasts and new small capillaries, extracellular matrix and insignificant number of inflammatory cells. No significant differences were identified in the individuals of the control group and those of the Honeyderm group.*

**Keywords:** *Hippophae rhamnoides, sea buckthorn oil, wound healing, external treatment*

### **Introduction**

Wounds have a wide classification depending on the aetiology, the timing of the injury and the depth. In most of the cases, secondary bacterial is associated and treating the wound can become a real challenge for veterinarians. The use of antibiotics should be taken in the last consideration and be replaced by naturally derived treatments. Phytotherapy has proved great results in human medicine and starts to be implemented in veterinary medicine since the last few years. An old known plant, sea buckthorn, has been used for centuries in humans and in animals for its nutritional values. Sea buckthorn oil has been the subject of some researches for external applications in humans for skin injuries and skin diseases such as in psoriasis condition (Boca et al., 2018) but also eczema and burns wounds (Zeb A., 2011). In animals, good results were obtained in sheep with burn wounds (Vinita et al., 2017). This multifaceted plant was used since the ancient time and is produced in variable forms for multiple therapeutical effects such as wound healing. Nowadays, sea buckthorn has turned out to be famous and known worldwide, particularly for its beneficial outcomes on human and animal organisms. Sea buckthorn is esteemed for its cell reinforcement and antioxidant effect, cardioprotective, antiatherogenic, antidiabetic, hepatoprotective, anti-carcinogenic, immunomodulating, antiviral, antibacterial, anti-inflammatory and vasodilating impacts. Its beneficial outcomes have been used for increasing the speed of wound healing (Krejcarová et al., 2015). Sea buckthorn has also beneficial effects on skin problems in humans, particularly on a common chronic dermatological condition known as psoriasis. It is clinically described as round, erythematous, well-delimited plaques, which can be covered by scales on the skin (Boca et al., 2018). Psoriasis also occurs in animals. Many cases have been reported in monkeys and dogs. Animal models represented by mice with psoriasis-like lesions, have been used for a better understanding of the disease (Rodríguez- Martínez et al., 2017). Sea buckthorn oil is a precious topical agent to apply on skin lesions.

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## Materials and methods

The aim of the present study was to evaluate the healing process by applying fatty oil extracted from sea buckthorn plant in comparison with Honeyderm, a product already used on the market in veterinary medicine. The main objective of this study was to evaluate the effect of sea buckthorn oil externally in comparison with Honeyderm product on created wounds treated by second intention healing. To conduct the present study, rabbits were used as model. Indeed, these animals have the advantages to possess good healing abilities in good maintenance conditions. They are also easy to handle and to manipulate every day in order to apply the treatments. We used 3 groups of rabbits to investigate sea buckthorn oil properties on wound healing. On each of the rabbits, we created surgically defects, or cutaneous wounds, on the cranial region of their back. The surgeries were performed at the Surgery Department of the Faculty of Veterinary Medicine of Cluj-Napoca. In this study, we wanted to compare sea buckthorn oil effects with a commercial product already approved on the market for skin healing. We chose a product in which the composition contain the most natural substances as possible, such as: Honey, hyaluronic acid, lavender HE, Aloe Vera, vitamin E and shea butter. Honeyderm is a yellowish paste mixture and is used for superficial wounds healing by secondary intention. For the extraction of the oil, we used relatively dried sea buckthorn, containing about 89 % dry substance. The fatty acid profile and the gross chemical composition were determined.

The objectives of the study included: evaluation of wound healing; major differences of wound healing using sea buckthorn oil and Honeyderm treatments on wounds; histological examination of the wounds treated by sea buckthorn oil and Honeyderm.

For the practical part, we used 3 groups of rabbits to investigate sea buckthorn oil properties on wound healing. On each of the rabbits, we created surgically defects, or cutaneous wounds, on the cranial region of their back. Two types of cutaneous wounds have been induced from either side of the vertebral column: on the left side, 4 round defects made by a biopsy trocar of 5 mm and on the right side 4 rhomboidal defects made by a surgical scalpel inducing larger defects.

In order to study the effect of sea buckthorn oil in comparison with Honeyderm product on second intention healing wounds, we needed to create standardized conditions. We started to apply treatments the day after the surgeries were done. The wounds were treated with sea buckthorn oil and Honeyderm without applying any other treatment on the wounds to limit any interference in the healing process. Q-tips were used to directly apply the treatments on the wounds. The control group was only treated with saline solution; the 2nd group received sea buckthorn oil on the first 4 cranial wounds and Honeyderm treatment on the last 4 caudal wounds, 1x per day only, while the last group was receiving treatments 2x per day, one in the morning and one in the evening. The wounds aspects were checked every day, by naked eyes, by the same person, in order to obtain a subjective evaluation of the wound healing. To assess the wounds, we appreciated first the appearance of granulation tissue into the wound, and the filling of the entire wound by granulation tissue. We were also attentive on the presence of any exudate into the wounds.

We observed that 14 days after the creation of the skin defects, complete healing of the wounds was obtained. And due so, after 13 days of treatment, we decided to do biopsies of healed wounds. We took a sample of 1cm of diameter from the control group, from wounds treated with sea buckthorn oil and from wounds treated with Honeyderm. Histological examinations were realized in order to evaluate the effect of sea buckthorn oil treatment on the healing process, at a microscopic point of view, and be compared with Honeyderm treatment and without treatment

(control). The tissue sections were evaluated for the degree of fibroblast proliferation, neoangiogenesis, inflammation, epidermis and total scar thickness.

### Results and discussions

The sea buckthorn fruits collected were chemically examined. Fruits had a dry substance content of 18.3 %. The value obtained ranges between the 15-20 %, limits given by most authors. The raw protein content of sea buckthorn is increased. It reaches or even exceeds the values found in the literature. The value is equal to or higher than the protein level of the sea buckthorn fruit. The value of the raw fat of sea buckthorn (34.44 % DM) is found within the gross fat limits indicated by the Romanian authors. We found that extracts of sea buckthorn oil contain large amounts of essential fatty acids, the first being vitamin F. The fact that the unsaturated fatty acids contained in sea buckthorn oil in large proportions explains the prophylactic and therapeutic action of sea buckthorn oil in various internal and external diseases.

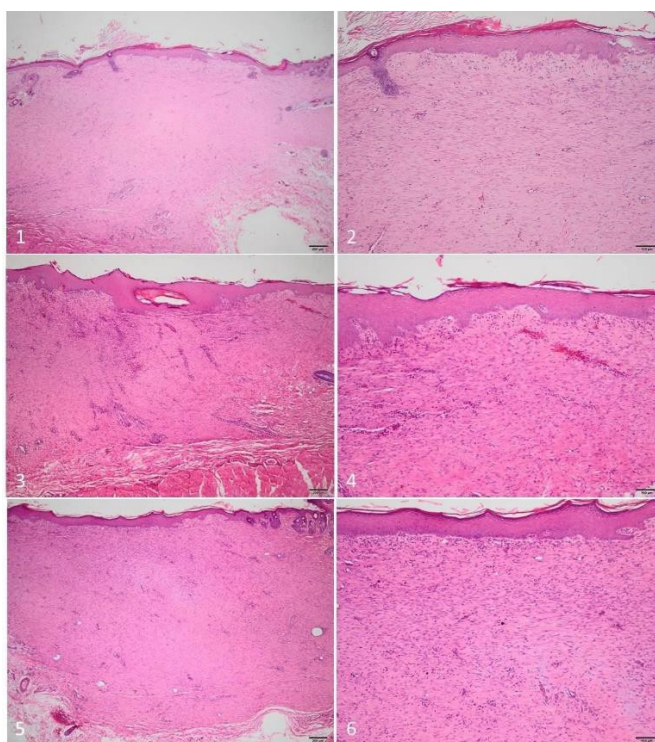


Fig. 1: Histological features of skin wound regeneration: 1 and 2) Control group; 3 and 4) Honeyderm group; 5 and 6) Oil group. Hematoxylin and eosin (H&E).

As an average, we observed a complete healing within 14 days after surgical creation of the cutaneous wounds. In control group (cutaneous defects and no treatment), the cutaneous defects were filled with a moderate amount of immature fibrous tissue composed by high numbers of fibroblasts, moderate extracellular matrix and few small blood vessels (neoangiogenesis) (Fig. 1-1).

Scattered inflammatory cells predominated by heterophils and macrophages were found within the perivascular area of the scar tissue. Total thickness of 104,88  $\mu\text{m}$ , and covered by small amounts of keratin lamellae (Fig. 1-2). In contrast to control group, no significant differences were identified in the individuals treated with Honeyderm (Fig. 1-3,4). In wounds treated with sea buckthorn oil group, the cutaneous defects were filled with an abundant amount of immature fibrous tissue composed of large number of fibroblasts.

Another popular treatment that proved good results in wound healing by secondary intention is propolis. Complete healing of the wounds has been obtained after 9 days of propolis treatment compared to sea buckthorn oil which was after 14 days. Histological examination demonstrates that propolis and sea buckthorn oil have anti-inflammatory actions on the treated wounds (Beteg F., 2018). Sea buckthorn oil was also topically applied on wounds of burnt sheep during 21 days after the injury. Determinations of the blood flow and epithelization were done every 6 days. A higher percentage of epithelization was observed in the wounds treated with sea buckthorn than in the untreated one (Vinita et al, 2017).

### Conclusions

The raw chemical composition of sea buckthorn dry fruit was characterized through values, which are very close to those one presented in the literature. The use of sea buckthorn oil as a product, with an original formula, for the treatment of some lesions characterized by tissular damages in rabbits, demonstrates good therapeutical results. Regarding our results on the healing quality of the wounds treated in our experiment, we can affirm that the best wound healing were those treated with sea buckthorn oil. Indeed, it gave us the greatest healing features at a microscopic point of view. Nevertheless, we have to take in consideration the licking habits of some of the individuals, which have probably delayed and slightly decreased the quality of the healing. By comparing histologically sea buckthorn oil and Honeyderm, we concluded that sea buckthorn oil provides scar tissue regeneration faster than Honeyderm with a higher proliferation of fibrous tissue and neoangiogenesis. Animal practices can benefit from the continuous research on sea buckthorn, as it represents an alternative treatment for many animal species and in different diseases. This plant is an economic and ecologic solution that is found worldwide. Sea buckthorn can be prepared in many ways, not only in products used for external treatments.

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